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Biostimulation of a novel native Bacillus strain capable of degrading gasoline

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ABSTRACT

The contamination of soil with hydrocarbons is a significant ecological issue worldwide, posing a threat to the ecosystem. Therefore, it is crucial to develop environmentally friendly and effective techniques to eliminate pollutants using biological agents. This study aimed to isolate, characterize, and evaluate bacteria capable of degrading gasoline. Soil samples contaminated with gasoline were collected and diluted up to 10-10. Pure cultures were obtained by streaking dilutions of 10-7, 10-6, and 10-5 on nutrient agar. These pure cultures were then inoculated on a selective medium (Bushnell Haas minerals agar). The isolates were characterized based on their growth patterns using morphological characteristics, optical density measurement, and biochemical tests. As a result, five gasoline-degrading bacteria were identified as *B. mojavensis* and B. licheniformis. Two biodegradation experiments were conducted using low and high concentrations of gasoline (0.01/10 and 0.001/10 mL, respectively) over a period of 14 days. Furthermore, the bacterial strains were stimulated by adding nitrogen and phosphorus to evaluate their efficiency in degrading gasoline. The results showed that *B. mojavensis* has the potential to degrade gasoline more effectively than the other isolate. Additionally, the addition of nitrogen minerals enhanced the ability of this isolate to degrade gasoline. Conversely, the performance of B. licheniformis in degrading gasoline was improved with the addition of nitrogen and phosphorus. This study demonstrates the effectiveness of Gram-positive bacteria in gasoline degradation and highlights the potential of the genus *B. mojavensis* for in situ remediation of gasoline-contaminated soils.

Contribution/Originality: No Previous research has specifically identified bacteria capable of degrading gasoline using Biostimulation. This study confirms that *Bacillus mojavensis* is efficient in degrading gasoline under controlled biostimulation conditions. Our findings will have significant implications for developing more efficient bioremediation technologies, for controlling petroleum pollution across various environmental ecosystems.

1. INTRODUCTION

Gasoline is a refined product of petroleum consisting of a mixture of Hydrocarbons, additives, and blending agents. It is common for groundwater and soil to be contaminated with gasoline or diesel fuel by leaking underground storage tanks and by accidental spills and leaks from pipelines. The compounds in these groups may be mobile and can cause significant damage not only to soil but also to water intakes and groundwater reservoirs [1]. These phenomena are mainly caused by human activities such as urbanization, industrialization, and

civilization. As a result, pollutants derived from hydrocarbons can be harmful to the immune system, cause genetic mutations, and lead to cancer in humans and animals, while also negatively impacting natural ecosystems [2].

There are several ways to mitigate hydrocarbon pollution. These include mechanical, chemical, and biological methods. Biological remediation (bioremediation) is a promising technology for the treatment of hydrocarbon-contaminated environments since it is cost-effective, efficient, and easy to use. However, it requires a long time to completely degrade pollutants [3].

The Biostimulation process enhances biodegradation by stimulating the growth of naturally occurring microorganisms that can break down hydrocarbons [4]. This involves adding nutrients, maintaining specific environmental conditions (such as pH, temperature, and aeration), and using surfactants to contaminated soils. This technique can be applied in situ and ex-situ [5] to enhance biodegradation by increasing the bioavailability of the pollutants and the growth rate of native (indigenous and autochthonous) hydrocarbon-degrading microorganisms [6].

Several studies have demonstrated that bacteria, fungi, yeasts, protozoa, and algae play a major role in transforming hydrocarbon pollutants into harmless compounds. However, bacteria are the most dominant and active degraders [7]. Most of the genera capable of degrading carbohydrates: Achromobacter, Marinobacter, Actinobacter, Alcaligenes, Mycobacterium, Arthrobacte, Bacillus, Rhodococcus, Corynebacterium, Micrococcus, Flavobacter, Nocardia, Bravibacterium, Streptococcus, Bacillus, Stenotrophomonas, Methylobacterium, Enterobacter, and Pseudomonas [8]. Despite this, there is limited data on the findings of the isolated indigenous gasoline degrader bacteria from contaminated soils.

In Sudan, gasoline stations, agricultural wastes, and industrial effluents are the main sources of pollutants that negatively affect soil and water quality. However, little is known about bacterial isolates native to Sudan's environment that degrade hydrocarbons. Hence, the objective of the study was to identify and characterize potential gasoline-degrading bacteria from gasoline-contaminated soil. In addition, the efficiency of these strains on the degradation of gasoline was evaluated using the Biostimulation process.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

Samples were collected from soil contaminated with gasoline from Nile station at 7:28 am in sterilized plastic containers. The soil samples were collected from different sites to a depth of 30 cm by hand digging. The soil was mixed thoroughly, sieved through screens with 2 mm diameter openings to remove stones, and other foreign materials. The material was kept in sterile polyethylene bags and stored at 10°C.

2.2. Isolation of the Isolates

One gram of the soil was weighed and dissolved in 10 ml distilled water. Serial dilutions up to 10^{-10} were made. The dilutions 10^{-7}, 10^{-6}, and 10^{-5} were streaked on nutrient agar. The inoculated plates were incubated at 37 C° for 48 hrs. A pure culture was prepared from all separate growing colonies with two replications for each.

2.3. Isolation of Gasoline Degrading Bacteria

To determine the ability of the isolates to degrade gasoline the isolates were grown on Bushnell Haas mineral salt (BHMS). In order to determine the optimum concentration of gasoline that enhances bacterial growth, different amounts of 0.001, 0,1, and 1mL of the gasoline were added to the Bushnell Haas Agar medium. The medium was incubated at 30C° for one week. Pure culture was obtained from single-growing colonies. The isolates were subjected to various morphological and physiological tests (Figure 1).

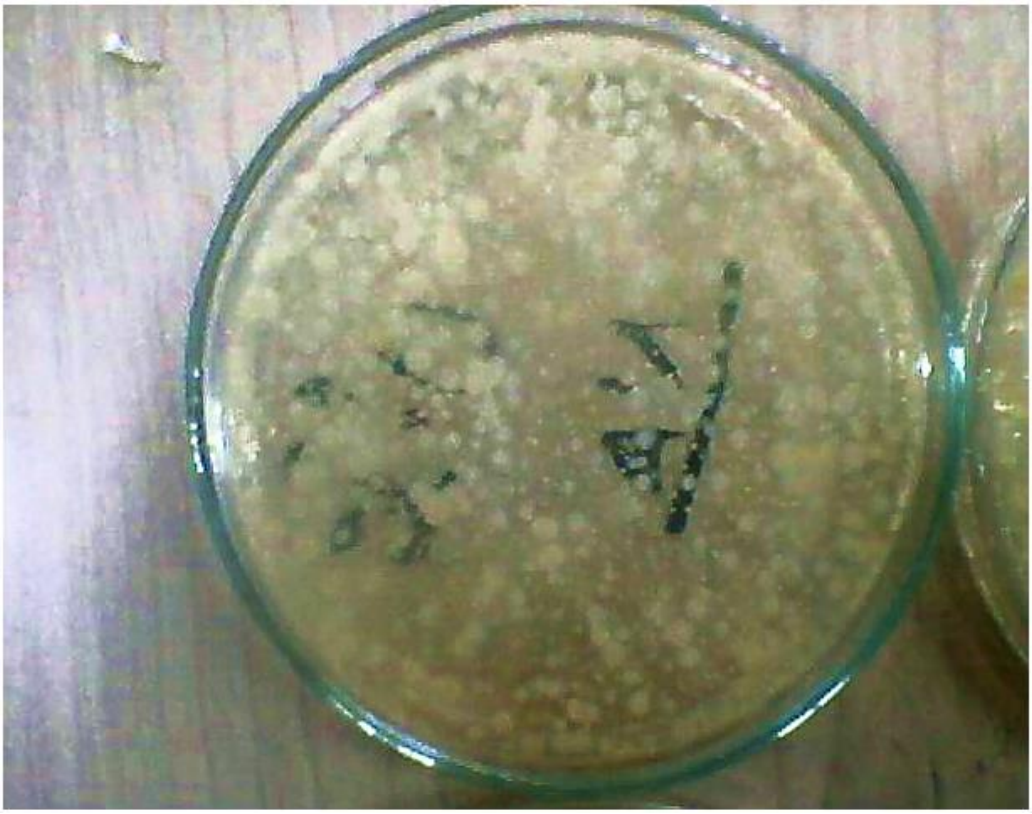


Figure 1. The bacteria growth on selective media (BHMS).

2.4. Bacterial Morphology and Biochemical Tests

Morphological Characteristics: A 24-hour culture was prepared from each isolate. Morphological Characteristics. The isolates' morphological characteristics were studied using Gram's staining and endosphere formation protocols and microscopy results.

Biochemical Tests: A 24-hour culture was prepared from each isolate. Catalase test, oxidase test, nitrate reduction, V.P test, hydrolysis of starch, growth in PH, growth in Nacl, Citrate utilization, indole test, Gelatine liquefaction, Production acid from mannitol, Glucose utilization test, Hugh and lefison medium test, Oxidation/fermentation (O/F) test, Methyl red (M R) test, Phenol red dextrose broth test, Phenol red lactose broth test, Oxygen molecular demand test were performed.

2.5. Evaluation of the Isolates to Degrade Gasoline

The evaluation of the isolates to degrade gasoline was carried out using the enrichment medium Bushnell Haas mineral salt (BHMS) agar medium. The medium contained $MgSO_4.7H_2O$ (0.2g/L), $CaCl_2$ (0.02g/L), KH_2PO_4(1g/L), K_2HPO_4 (1g/ L), and NH_4NO_3(1g/L) and 2 drops from 60% of $FeCl_3$ at pH 7.2. The medium was distributed in test tubes each tube containing 10 ml. The tubes were divided into sets. Each of the tubes of the first set receives 0.1ml and each of those of the second set receives 0.001ml of the gasoline. Each tube was inoculated with 1 ml of 24-hour-old isolate suspension in nutrient broth. Each treatment was replicated three times. The tubes were incubated in the shaker at room temperature for two weeks.

The growth (Turbidity) was measured using a UV spectrophotometer at 595nm in replicate from zero time - 14 days at regular intervals of one week against BHMS medium as a blank.

2.6. Effect of Nitrogen and Phosphorus Minerals on Degradation Bacteria (Bio Stimulation)

Nitrogen and phosphorus minerals were used to enhance the growth of the bacterial isolates. Ten mL of medium Bushnell Haas mineral salt (BHMS) were distributed in test tubes. In one set each tube receives 0.1 mL of

gasoline and each of those in the second set receives 0.001 mL of gasoline as a carbon source. Each of the tubes was inoculated with 1 ml of 24-hour bacteria suspension in nutrient broth. Then, the different mineral treatments were distributed as follow:

1- Nitrogen minerals (0.025g of ammonium nitrate) were added to each tube with two replications.

2- Phosphorus minerals (1.0 g of di potassium hydrogen phosphate) were added to each tube with two replications.

3- Phosphate and nitrogen minerals (1.0g of di potassium hydrogen phosphate with 0.025g of ammonium nitrate) were added together to each tube with two replications. The tubes were incubated in the shaker at room temperature for two weeks. The growth (turbidity) was measured using a UV spectrophotometer at 595nm. The results were recorded at zero-time, one week and two weeks against BHMS medium as a blank.

3. RESULTS

3.1. Biochemical Characterization

Five bacterial isolates that are capable of degrading Gasoline were obtained. Table 1 shows the results of the different biochemical tests. The microbiological investigations showed that the five isolates were gram-positive, motile, and spore-forming. They are also catalase-positive, and oxidase-positive and produce acid from sucrose and glucose. They hydrolyzed starch and gelatine. The isolates 1,2,4,5 were aerobic while isolate 3 was facultative and did not produce acid from methyl red (Figure 2). *According to these tests the isolates were identified as *Bacillus mojavensis* and *Bacillus licheniformis*.

Table 1. Morphological, biochemical and physiological characteristics of bacterial isolates isolated from gasoline contaminated soils.

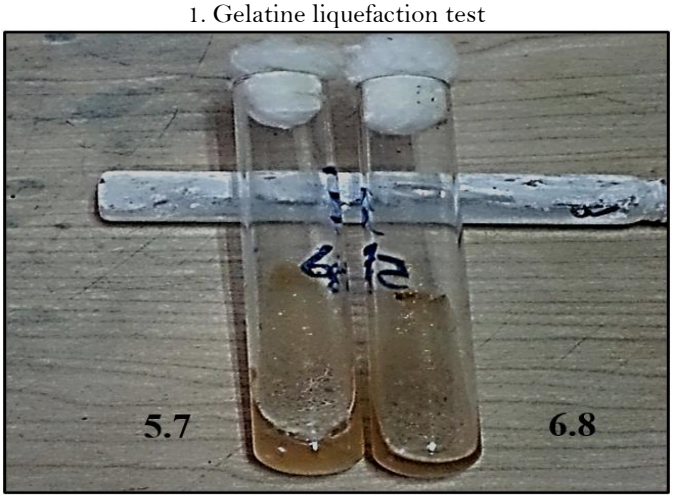
Character	Isolation				
	1	2	3	4	5
Shape	Rod	Rod	Rod	Rod	Rod
Gram stain	+	+	+	+	+
Endo spore stain	+	+	+	+	+
Oxygen molecular demand	Aerobic	Aerobic	Anaerobic facultative	Aerobic	Aerobic
Fermentation	+	+	+	+	+
Oxidation/fermentation (O/F) test	O	O	O	O	O
Catalase test	+	+	+	+	+
Oxidase test	+	+	+	+	+
Motility test	+	+	+	+	+
Hydrolysis of:					
1- Starch	+	+	+	+	+
2- Gelatin	+	+	+	+	+
Production acid from:					
1- Sucrose	+	+	+	+	+
2- Glucose	+	+	+	+	+
Methyl red	+	+	-	+	+
Vp test	+	+	+	+	+
Indole test	+	+	+	+	+
Citrate acid	+	+	+	+	+
Mannitol test	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Urease test	-	-	-	-	-
Growth in concentration of NaCl					
1- 2%	+	+	+	+	+
2- 5%	+	+	+	+	+
3- 7%	+	+	+	+	+
4- 10%	+	+	+	+	+
Growth in PH					
1- 5.7	+	+	+	+	+
2- 6.8	+	+	+	+	+

Control Isolates

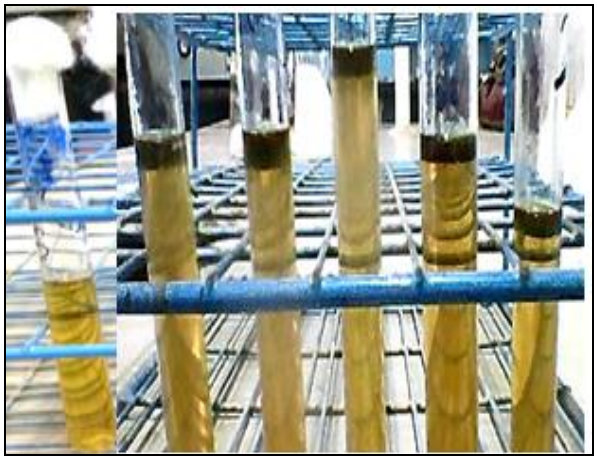
1. Gelatine liquefaction test

Control Isolates

2. Citrate utilization test

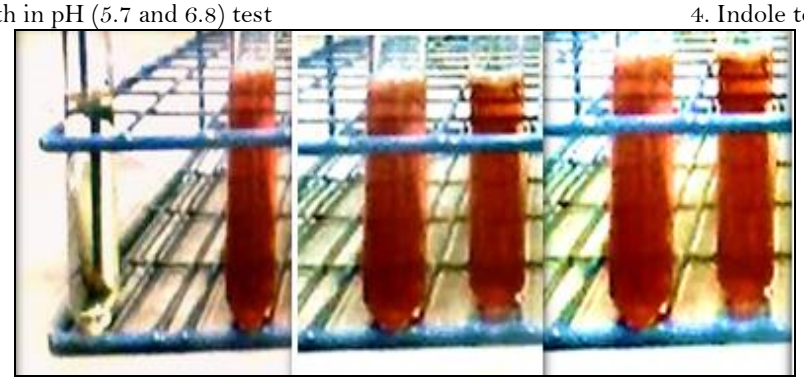


3. Growth in pH (5.7 and 6.8) test



Control Isolates

4. Indole test



Control Isolates

5. Nitrate reduction test

Figure 2. Some of biochemical tests on isolates.

3.2. Evaluation of the Isolates to Degrade Gasoline

0.001/10mL and 0,1mL/10mL of the gasoline concentrations were optimal for the growth of the bacteria. While there is no bacterial growth was detected in the medium containing 1mL/10mL of the gasoline concentration. This result indicated that the high level of gasoline inhibits the growth of the bacterium.

Figure 3 shows the growth of the two bacterial species in Bushnell Haas mineral alt (BHMS) medium containing (0.01ml/10ml) gasoline as a source of carbon. The growth of the species *Bacillus mojavensis* was increased till it reached the peak after 7 days (0.115nm). Then started to decline sharply. On the other hand, the species *Bacillus licheniformis* showed a slight increase in growth after 7 days.

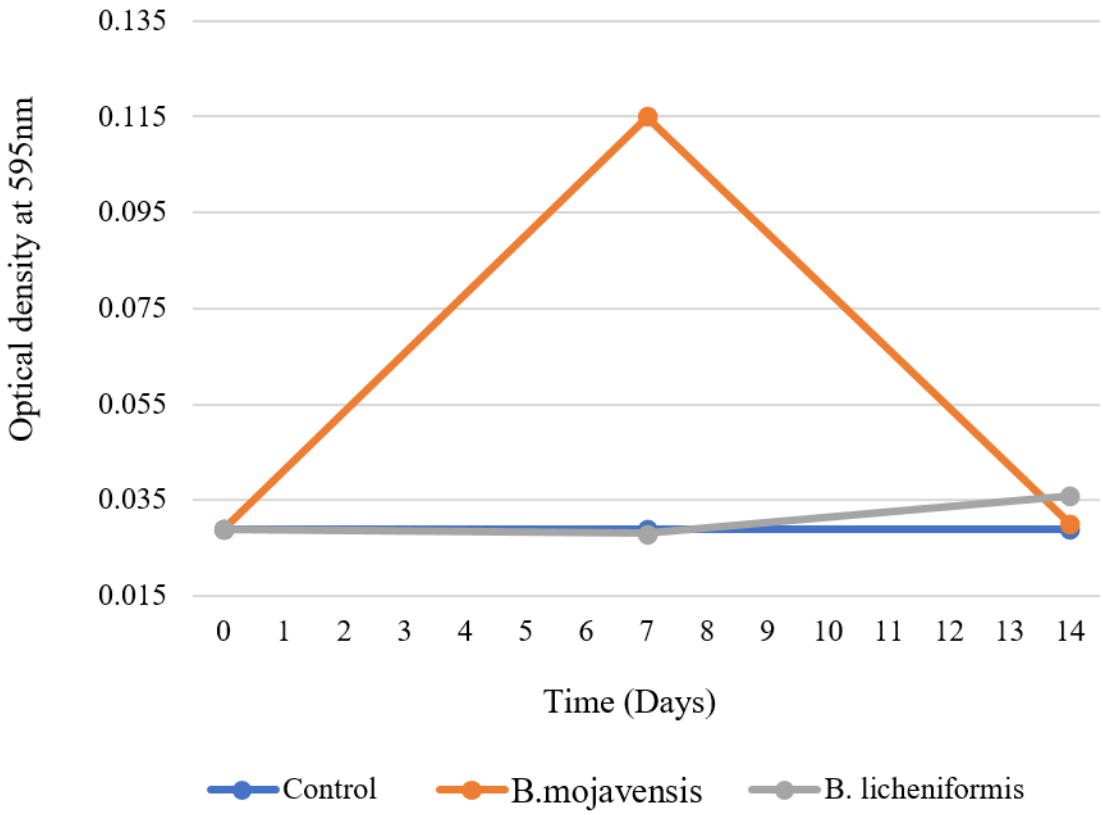


Figure 3. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium according to (O.D value) at 595 nm with (gasoline concentration 0.01ml/10ml) for 14 days of incubation against BHMS medium as control.

The growth of *Bacillus mojavensis* showed a continuous linear growth with time in the BHMS medium containing 0.001mL/10mL (Figure 4). The Bacillus licheniformis showed a rapid rise in growth where it reached the maximum after 7 days. Then started to decline till 0.034 nm after 14 days.

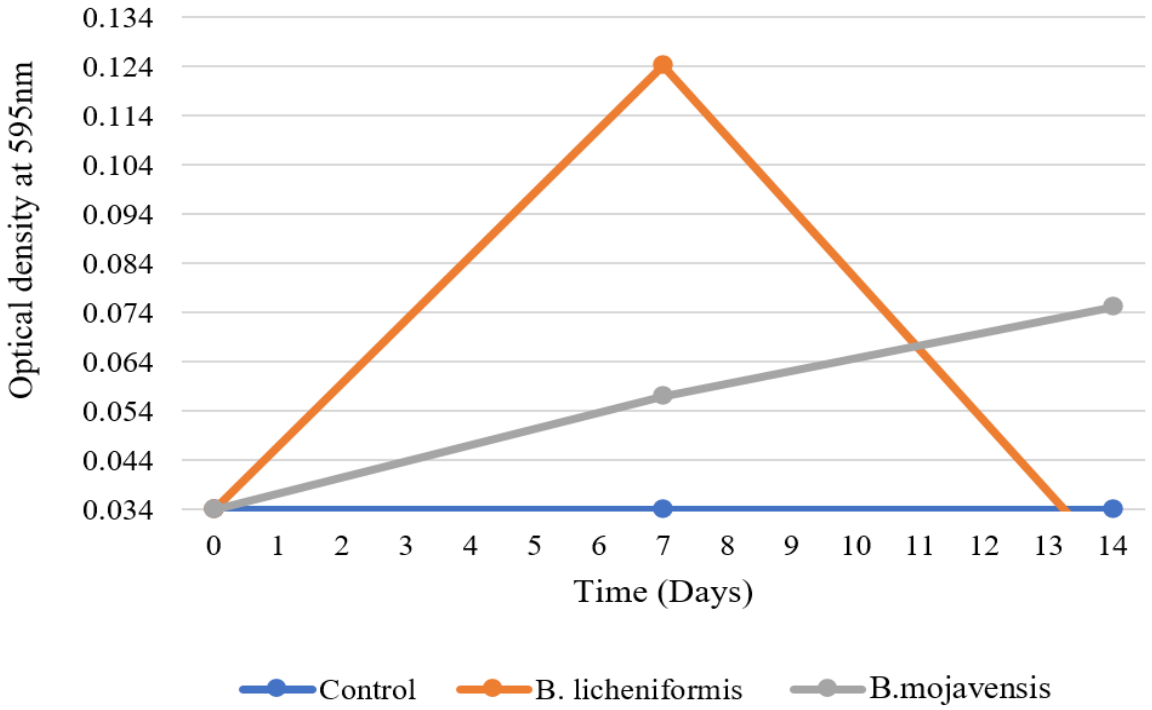


Figure 4. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium according to (O.D value) at 595 nm with (gasoline concentration 0.001ml/10ml) for 14 days of incubation against BHMS medium as control.

3.3. Effect of Nitrogen and Phosphorus Minerals on Degradation Bacteria (Bio Stimulation)

Figure 5 indicates the growth of the two bacterial species in BHMS medium containing 0.01mL/10mL gasoline and 0.02g nitrogen. The addition of nitrogen resulted in a continuous rise in the growth of Bacillus mojavensis where it continues to grow linearly with time till 14 days (0.145 O. D). The growth of Bacillus licheniformis increased with time till it reached the peak in 7 days (0.065 O. D) and then declined. Figure 6 shows the growth of both Bacillus species in a BHMS medium containing 0.001mL gasoline and supplemented with 0.2g nitrogen. Again, Bacillus mojavensis kept a continuous linear growth with time till reached 0.045 O. D in 14 days. The species Bacillus licheniformis was suppressed by the addition of nitrogen where no rise in growth was observed.

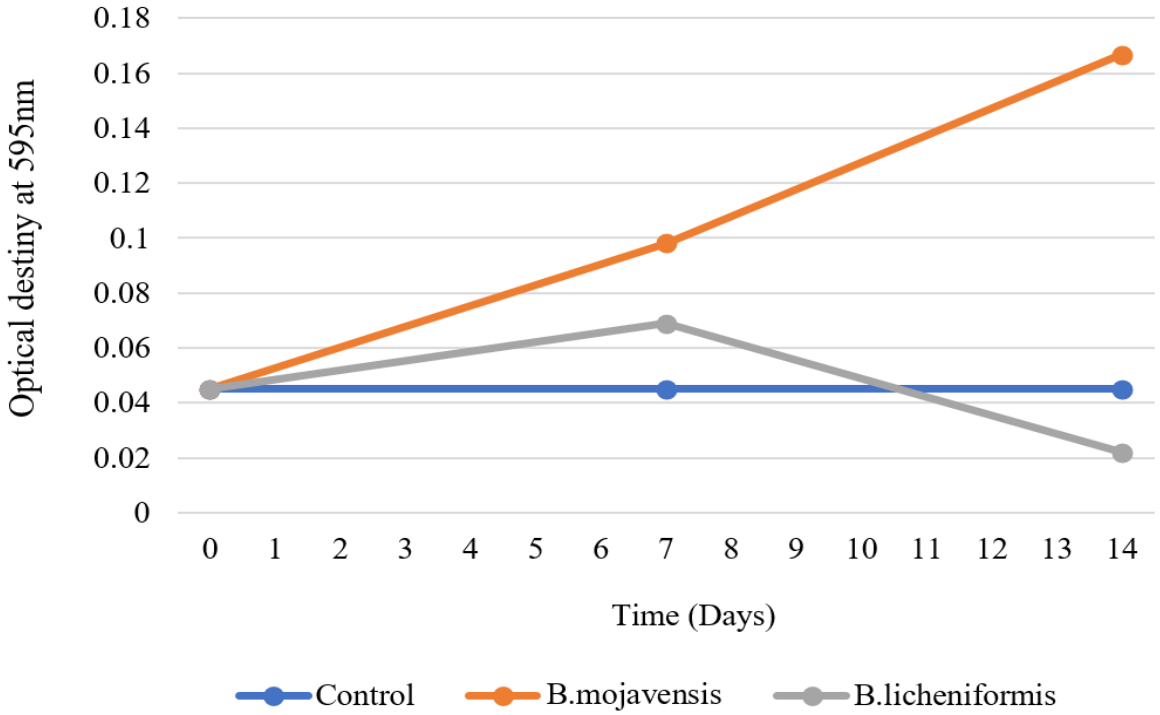


Figure 5. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with nitrogen mineral a ccording to (O.D value) at 595 nm (Gasoline concentration 0.01ml/10ml) for 14 days of incubation against BHMS medium as control.

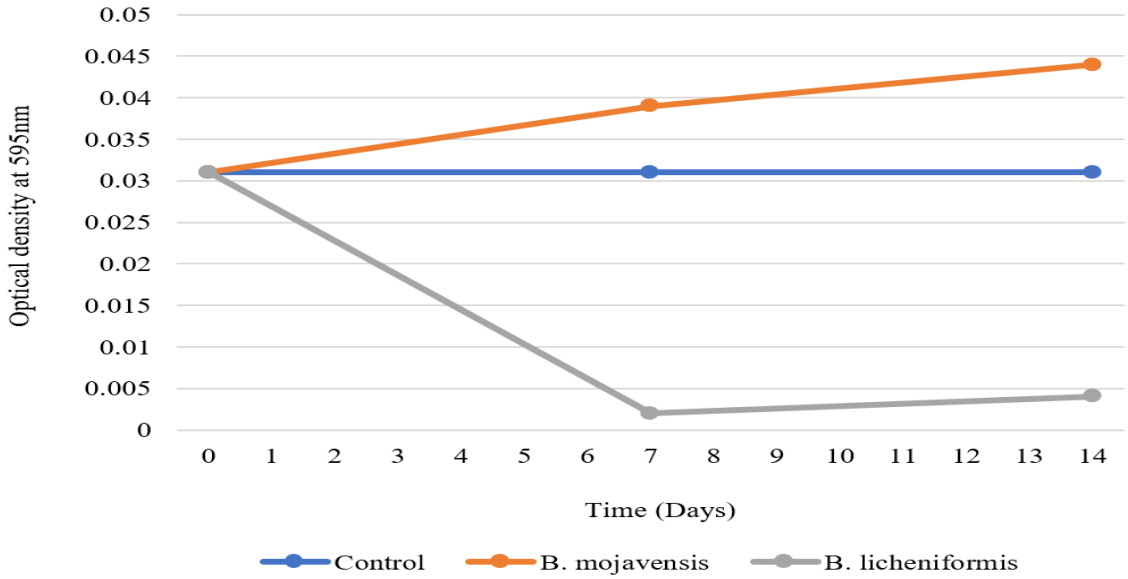


Figure 6. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with nitrogen mineral a ccording to (O.D value) at 595 nm (gasoline concentration 0.001ml/10ml) for 14 days of incubation against BHMS medium as control.

Figure 7 reveals the growth of the bacterial species in a BHMS medium containing 0.01mL gasoline and supplemented with 1.00g phosphorus. The addition of phosphorus enhances the utilization of gasoline by the species Bacillus licheniformis where it showed rapid rise in growth and reached its peak in 7 days and then started to decline. On the other hand, the addition of phosphorus suppressed the growth of Bacillus mojavensis in high concentrations of gasoline where it showed linear lower growth. Figure 8 indicates the growth of the bacterial species in a BHMS medium containing 0.001mL gasoline and supplemented with 1.00g phosphorus. In this case, the growth of the two bacterial species was reversed. The addition of phosphorus to the lower concentration of gasoline enhanced the growth of Bacillus mojavensis where it reached its maximum in 7 days and then sharply declined. The growth of Bacillus licheniformis assumed the same pattern of growth of Bacillus mojavensis in the high concentration of gasoline with phosphorus.

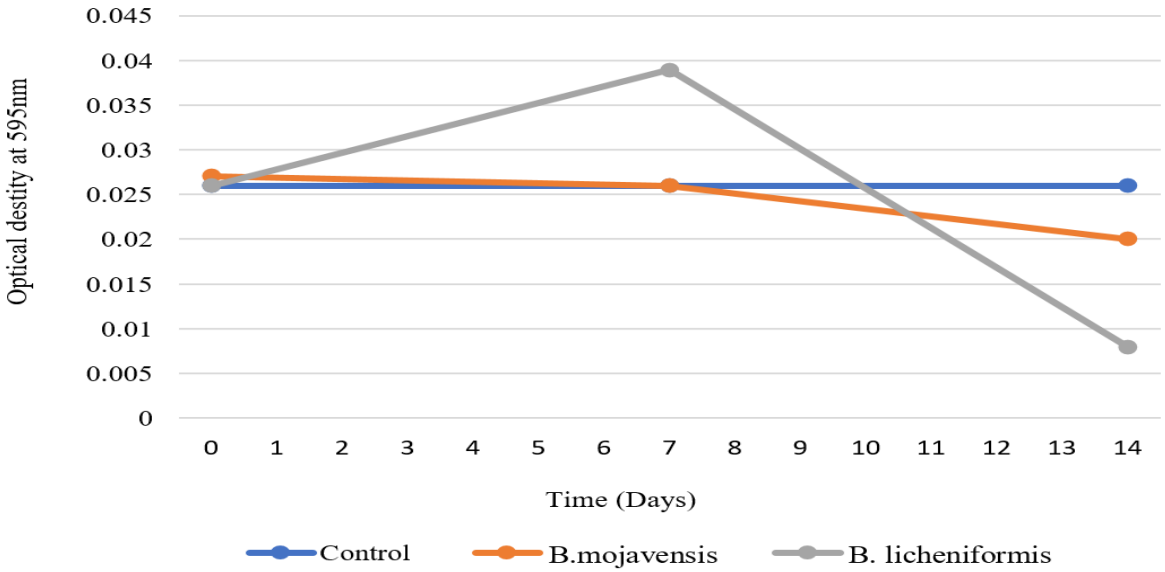


Figure 7. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with phosphorus mineral a ccording to (O.D value) at 595 nm (Gasoline concentration 0.01ml/10ml) for 14 days of incubation against BHMS medium as control.

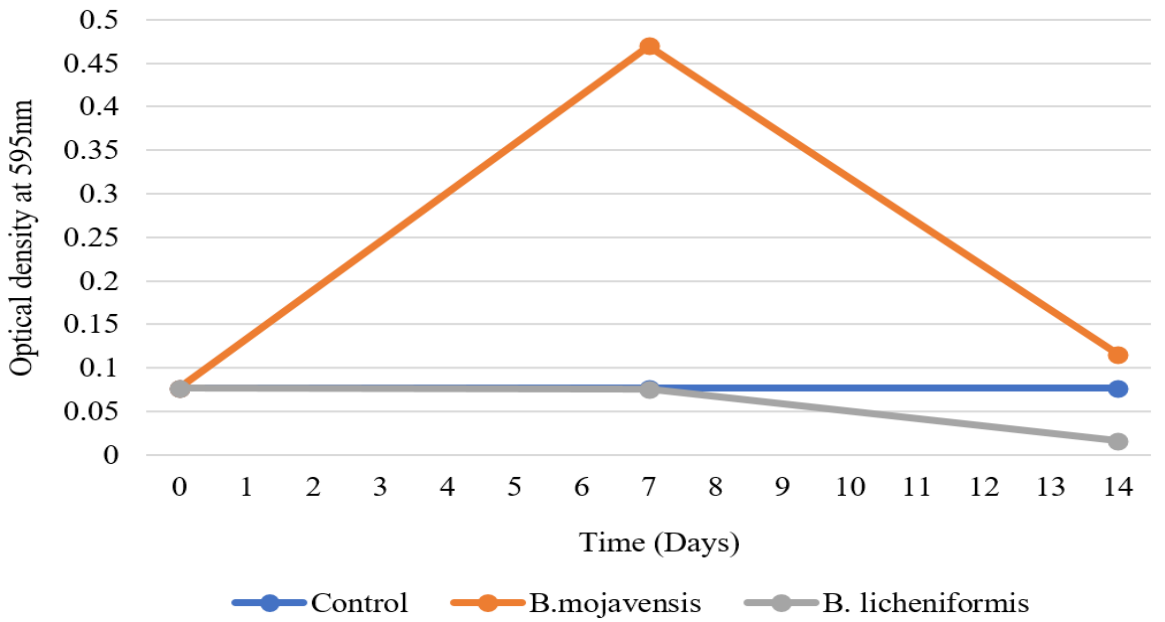


Figure 8. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with phosphorus mineral a ccording to (O.D value) at 595 nm (Gasoline concentration 0.001ml/10ml) for 14 days of incubation against BHMS medium as control.

Figure 9 The supplementation of the BHMS medium containing 0.01mL gasoline 0.02g nitrogen and 1.00g phosphorus gave a growth of Bacillus mojavensis similar to its growth in the media containing only gasoline. Its growth reached its peak in 7 days and then declined. Concerning Bacillus licheniformis no growth was observed.

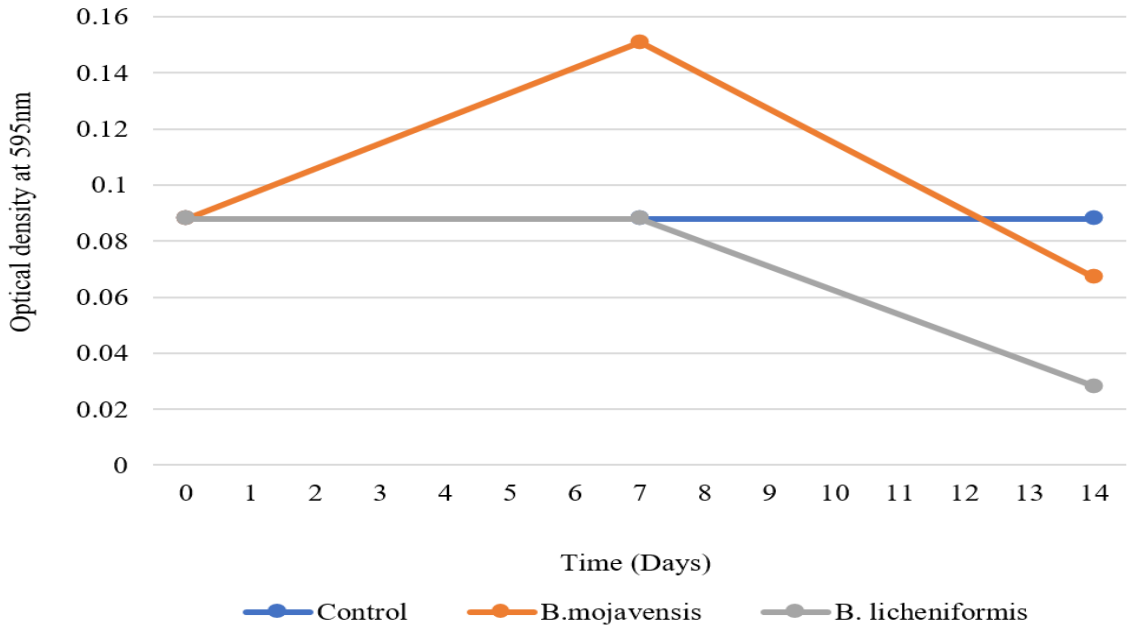


Figure 9. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with nitrogen and phosphorus minerals according to (O.D value) at 595 nm (Gasoline concentration 0.01ml/10ml) for 14 days of incubation against BHMS medium as control.

The addition of both nitrogen and phosphorus to the medium containing 0.001mL gasoline resulted in the enhancement of the growth of the species Bacillus licheniformis where the growth continued to rise till the end of the incubation period. On the other hand, Bacillus mojavensis showed no rise in growth up to the 7th day then started to increase in growth till the end of the incubation period (Figure10).

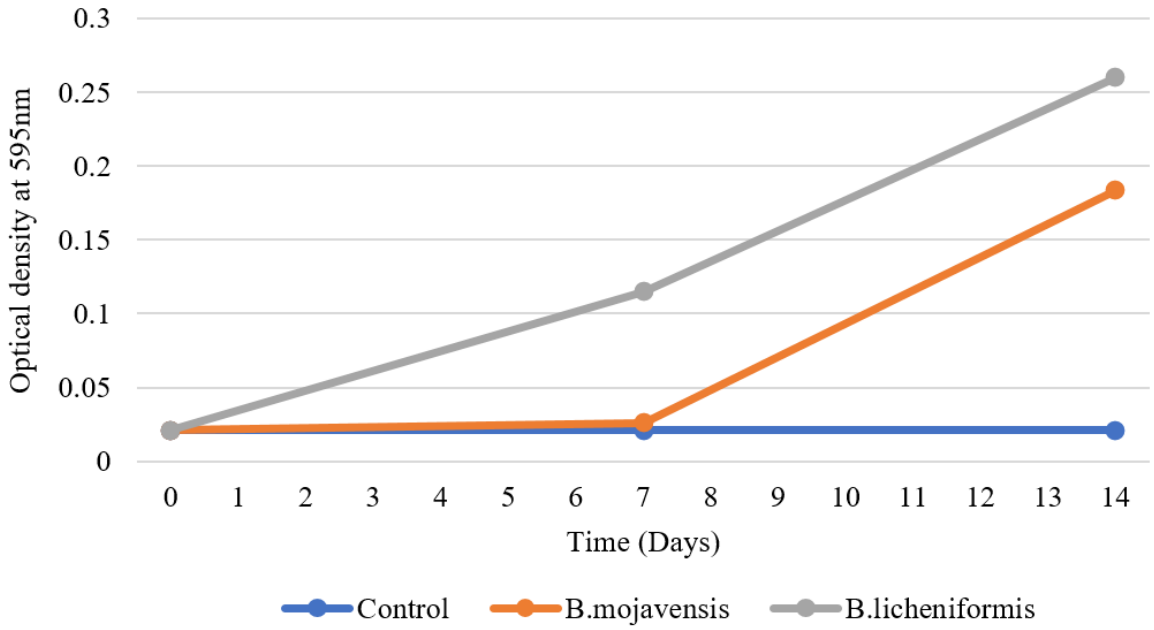


Figure 10. Growth of *B.mojavensis* and *B.Licheniformis* in BHMS medium supplemented with nitrogen and phosphorus minerals according to (O.D value) at 595 nm (Gasoline concentration 0.001ml/10ml) for 14 days of incubation against BHMS medium as control.

4. DISCUSSION

Microorganisms play a major role in the biodegradation (bioremediation) of hydrocarbon pollutants in polluted soil [9]. In this study, gasoline-contaminated soil bacteria were isolated using a BHMS medium supplemented with 0.01 and 0.001 mL gasoline as a carbon source to determine their growth pattern and degradation potential. In this study, all isolates identified as gasoline degraders were Gram-positive. The isolates were also characterized using some standard biochemical tests based on their catalytic activities. The isolates were identified as belonging to the genus Bacillus, within which two species were identified, Bacillus mojavensis and Bacillus licheniformis. All species showed positive results for the degradation of hydrogen peroxide, casein, starch, urea, and acid production. This could be a preliminary indication that the isolates have diverse enzymes for catalyzing the degradation of various and/or specific substrates. Other related studies also confirmed that *Bacillus. sp* was also isolated from a wide variety of hydrocarbon-contaminated soils [10]. From the current and other previous studies, it could be recognized that *Bacillus. sp* is potentially obtained from various soil environments, mainly due to its ubiquity in terms of its diverse metabolic capability for hydrocarbon degradation.

The growth capacity of the species was then detected at different gasoline concentrations 0.1mL/10mL and 0.01/10mL. Gasoline degradation in both treatments showed a different trend depending on the C concentration. The growth of *Bacillus mojavensis* was rapid in the higher concentration than that of Bacillus licheniformis. This indicates that Bacillus mojavensis is more efficient in utilizing gasoline as a carbon source. The lower concentration of gasoline didn't enhance the growth of Bacillus mojavensis in the same manner but enhanced the growth of *Bacillus licheniformis* in the lower concentration. It is obvious that the two species alternative greatly in their ability to degrade gasoline.

The stimulation of the growth of the species was performed using nitrogen and phosphorus. Nutrients such as nitrogen and phosphorus can be limiting factors affecting the biodegradation process. The addition of nitrogen in the higher concentration of gasoline enhances the growth of *Bacillus mojavensis* when the growth continues in a rising manner till the end of the incubation period. The addition of the nitrogen most probably enhanced the efficient use of the carbon source by *Bacillus mojavensis*. The growth of *Bacillus licheniformis* in the medium containing a high concentration of gasoline supplemented with nitrogen was rapid, the growth reached the peak in a short period then declined sharply. The addition of nitrogen enhanced the utilization of the carbon by this bacterium. The growth of this bacterium in the lower concentration of gasoline was generally less than that in the high concentration of gasoline supplemented with nitrogen. This may be due to an appropriate ratio of C:N for biodegradation. In general, the molar ratios C: N ranging from 14:1 to 560:1 have been proposed as a suitable or optimum for biodegradation [11].

The addition of phosphorus in the high concentration of gasoline inhibited the growth of the *Bacillus mojavensis*. It seems that phosphorus makes gasoline as a source of carbon unavailable for the bacterium or may be due to the high concentration of phosphorus than the optimal concentration that is necessary for biodegradation. Reduction of hydrocarbon degradation rates due to excess nutrients was also reported by Walworth, et al. [12]. No phosphorus addition was necessary, as the phosphorus concentration in the media was sufficient to maintain the optimal ratio of carbon: nitrogen: phosphorus (C:N:P). In contrast to *Bacillus licheniformis* whose growth was enhanced by the addition of phosphorus for a certain period and then declined. With time both bacterial species showed declined growth. As we discussed above, the species Bacillus mojavensis needs a high amount of carbon for growth, while, the other species Bacillus licheniformis needs a low amount of carbon for growth. Thus, it seems that the addition of phosphorus inhibits the carbon utilization by this bacterium.

With the addition of phosphorus in gasoline at lower concentrations, the bacterium *Bacillus mojavensis* attained similar growth to that in gasoline at high concentrations. It's clear that the phosphorus enhanced the utilization of gasoline at lower concentrations by this bacterium. The growth of *Bacillus licheniformis* was inhibited by the addition of phosphorus. This may be due to the higher concentration of the phosphorus than the optimal; C:P ratio

was not appropriate for biodegradation of gasoline. In general, a C/N/P ratio of 120/10/1 [mg] is considered optimal for the growth of bacteria in the presence of contaminants [13]. It is likely that the two bacteria have different metabolic pathways for the utilization of gasoline in the presence of phosphorus.

When both nitrogen and phosphorus were added together to the high concentration of gasoline, Bacillus mojavensis exhibited a similar growth pattern when grown in gasoline alone. This demonstrated that both nitrogen and phosphorus are unavailable for the bacterium; they may antagonize each other. With regards to the growth of the bacterium *Bacillus licheniformis* in the high concentration, there is no growth was detected; it seems that the addition of phosphorus and nitrogen inhibited the utilization of carbon as a source of energy by this bacterium. The reduction of hydrocarbon degradation rates due to excess fertilization such as nitrogen and phosphorus was also reported by Walworth, et al. [14]. When both nitrogen and phosphorus were added to the lower concentration of gasoline, the growth of *Bacillus mojavensis* was enhanced after one week of incubation. While the bacterium *Bacillus licheniformis* fairly utilized gasoline in the presence of nitrogen and phosphorus. In comparison to its growth in gasoline with nitrogen, phosphorus enhanced the growth; In the presence of phosphorus, it can utilize gasoline as a carbon source.

5. CONCLUSION

This study showed that two species of Bacillus, Bacillus mojavensis and Bacillus licheniformis isolated from contaminated soil were able to degrade gasoline. This paper describes the first study on the isolation and characterization of gasoline-degrading bacterium from Sudanese soils. *Bacillus mojavensis* has the potential to degrade gasoline compared to other isolates. Addition of the nitrogen to this species stimulates the biodegradation of gasoline. From the data presented in this study, it can be concluded that the investigated strain Bacillus mojavensis could be considered a good bioagent for its application in the biodegradation of soil-contaminated gasoline and removing pollutants from the environment.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing-original draft, writing review and editing, visualization, project administration, G.A.M.; conceptualization, validation, investigation, resources, supervision, data curation, writing-review and editing, project administration, M.A.O.; methodology, investigation, visualization, project administration, S.B. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1] J. L. Gallego, J. Loredo, J. F. Llamas, F. Vázquez, and J. Sánchez, "Bioremediation of diesel-contaminated soils: evaluation of potential in situ techniques by study of bacterial degradation," *Biodegradation*, vol. 12, no. 5, pp. 325-335, 2001.

[2] K. Prathyusha, Y. Jagan Mohan, S. Sridevi, and B. Sandeep, "Isolation and characterization of petroleum hydrocarbon degrading indigenous bacteria from contaminated sites of Visakhapatnam," *International Journal of Advanced Research*, vol. 4, no. 3, pp. 357-362, 2016.

[3] A. Horel and S. Schiewer, "Microbial degradation of different hydrocarbon fuels with mycoremediation of volatiles," *Microorganisms*, vol. 8, no. 2, p. 163, 2020. https://doi.org/10.3390/microorganisms8020163

[4] H. Haller, A. Jonsson, J. Ljunggren, and E. Hedenström, "Appropriate technology for soil remediation in tropical low-income countries-a pilot scale test of three different amendments for accelerated biodegradation of diesel fuel in Ultisol," *Cogent Environmental Science*, vol. 6, no. 1, p. 1754107, 2020. https://doi.org/10.1080/23311843.2020.1754107

[5] M. L. Feitosa *et al.*, "Tracking mangrove oil bioremediation approaches and bacterial diversity at different depths in an in situ mesocosms system," *Frontiers in Microbiology*, vol. 10, p. 2107, 2019. https://doi.org/10.3389/fmicb.2019.02107

[6] G. O. Adams, P. T. Fufeyin, S. E. Okoro, and I. Ehinomen, "Bioremediation, biostimulation and bioaugmention: A review," *International Journal of Environmental Bioremediation & Biodegradation*, vol. 3, no. 1, pp. 28-39, 2015.

[7] R. Jayanthi and N. Hemashenpagam, "Optimization of BH medium for efficient biodegradation of Benzene, Toluene and Xylene by a Bacillus cereus," *International Journal of Current Microbiology and Applied Sciences*, vol. 4, no. 10, pp. 807-815, 2015.

[8] G. Kebede, T. Tafese, E. M. Abda, M. Kamaraj, and F. Assefa, "Factors influencing the bacterial bioremediation of hydrocarbon contaminants in the soil: Mechanisms and impacts," *Journal of Chemistry*, vol. 2021, no. 1, p. 9823362, 2021. https://doi.org/10.1155/2021/9823362

[9] A. Ayangbenro, "Biodegradation of natural bitumen by Providencia stuartii isolated from heavy oil contaminated soil," *Global NEST Journal*, vol. 19, no. 2, pp. 353-358, 2017. https://doi.org/10.30955/gnj.002148

[10] G. K. Bekele *et al.*, "Isolation and characterization of diesel-degrading bacteria from hydrocarbon-contaminated sites, flower farms, and soda lakes," *International Journal of Microbiology*, vol. 2022, no. 1, p. 5655767, 2022. https://doi.org/10.1155/2022/5655767

[11] H. Ouriache, I. Moumed, J. Arrar, A. Namane, and H. Lounici, "Influence of C/N/P ratio evolution on biodegradation of petroleum hydrocarbons-contaminated soil," *Algerian Journal of Environmental Science and Technology*, vol. 6, no. 4, pp. 2437-1114, 2020.

[12] J. Walworth, A. Pond, I. Snape, J. Rayner, S. Ferguson, and P. Harvey, "Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil," *Cold Regions Science and Technology*, vol. 48, no. 2, pp. 84-91, 2007. https://doi.org/10.1016/j.coldregions.2006.07.001

[13] S. C. Wilson and K. C. Jones, "Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): A review," *Environmental Pollution*, vol. 81, no. 3, pp. 229-249, 1993. https://doi.org/10.1016/0269-7491(93)90206-4

[14] J. Walworth, C. Woolard, J. Braddock, and C. Reynolds, "Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: An approach to determining optimum levels," *Soil and Sediment Contamination*, vol. 6, no. 5, pp. 465-480, 1997. https://doi.org/10.1080/15320389709383580